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Expression of the human renin gene in transgenic mice throughout ontogeny

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Abstract. Expression of a human renin genomic DNA clone extending 900 base pairs upstream and 400 base pairs downstream of the gene has been previously examined in adult transgenic mice. In adults, expression of human renin was evident in kidney, reproductive tissues, adrenal gland and lung. Previous studies of mouse and rat renin have demonstrated that kidney renin becomes evident at approximately 15 days of gestation and that expression is localized first to smooth muscle cells of the developing renal arterial tree and becomes progressively restricted to juxtaglomerular cells. As a prelude to performing cell specificity studies to elucidate the pattern of human renin gene expression in the developing kidney, 15.5 and 17.5 days of gestation fetuses and newborns were obtained for expression analysis. Tissues were pooled and expression was examined in kidney, liver, gastrointestinal (GI) tract, lung, heart and brain. The number of transgenic fetuses in each pool was determined by human renin-specific polymerase chain reaction of DNA purified from placenta or tail biopsies. Renal human renin expression was abundant at all three time points. Expression was also evident in the GI tract at 15.5 and 17.5 days of gestation. Interestingly, although no human renin mRNA was evident in lung at 15.5 or 17.5 days of gestation, extremely high levels of human renin mRNA were detected in the newborn lung. Expression of the human renin gene in these tissues was further confirmed by differential primer extension analysis which is capable of differentiating the closely related human and mouse renin messages. These transgenic mice should provide an interesting model to examine the expression and regulation of the human renin gene during kidney development.

Key words: Transgenic mice – Renin – Northern blot – Polymerase chain reaction – Primer extension

Introduction

The tissue and cell specificity of rodent renin expression has been extensively investigated and reported [1–8]. In contrast, relatively little is known about the expression of the human renin gene. Our lack of information presumably results from a scarcity of fresh human tissues needed for expression studies as well as the nearly complete lack of suitable human renin-expressing cell lines. Recently, we and others have utilized transgenic animals containing the human renin gene to examine its expression and regulation as well as its role in the pathogenesis of hypertensive cardiovascular disease [9–13]. Transgenic animals provide an excellent and novel model for examining the expression of the human renin gene, because these animals express human renin in a tissue-specific and cell-specific fashion. Correct species-specific expression patterns of the human renin gene were also observed in transgenic mice [9]. In addition, because human renin does not cleave rodent angiotensinogen appreciably, the regulation of the transgene can be studied without disturbing the physiological state of the animal [14, 15]. The role of the transgene in hypertension can then be determined by the addition of a suitably species-specific substrate (human angiotensinogen), either by intravenous infusion or by the generation of doubly transgenic animals containing both genes [13].

In our previous studies, transgenic mice were obtained which expressed a human renin genomic clone containing all exons and introns and extending approximately 900 base pairs (bp) upstream and 400 bp downstream of the gene [9]. The tissue- and cell-specific expression of the transgene was examined in a number of tissues obtained from adult animals. Human renin mRNA was clearly detected in kidney, adrenal gland, ovary, testes, adipose tissue and lung, but not in liver, heart or brain. In kidney, human renin mRNA was restricted to juxtaglomerular cells, and in adrenal gland was localized to pockets of cells in the outer adrenal cortex. Expression of human renin mRNA in kidney increased after oral administration of angiotensin converting enzyme inhibitor and active human renin was released into the systemic circulation. Herein we describe one of these human renin transgenic lines and the expression of human renin during late mouse development and in newborn mice.

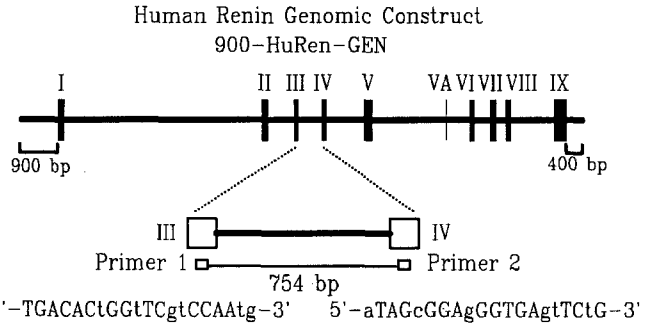

Fig. 1. Schematic map of the human renin transgene used in these studies. Exons are indicated with roman numerals. An expanded view of exon III, intron C and exon IV is shown along with the sequence of the oligonucleotide primers used in the polymerase chain analysis (PCR) analysis of Fig. 2. *Upper case letters* are nucleotides held in common with the endogenous mouse renin sequence while the *lower case letters* are those unique to the human renin sequence

Materials and methods

Generation of transgenic mice. The transgene utilized consists of a human renin genomic clone extending approximately 900 bp 5′ and 400 bp 3′ of the coding region (Fig. 1), as previously described [9]. This segment contains all ten exons (I–IX, VA) and all intervening intron sequences. The human renin portion of the clone was purified away from the vector sequences by *Bg*/II digestion and agarose gel electrophoresis, and cleaned for microinjection using Geneclean (Bio101). The purified fragment was microinjected into fertilized one-cell embryos derived from C57BL/10Ros X C3H/HeRos (BCF_2) at a concentration of 1.0 μg/ml. All mice used in this study are from the HuRen 9 transgenic line, an independent single line previously characterized with respect to HuRen expression in adult tissues.

All mice were fed standard mouse chow and water ad libitum. For captopril treatment, the inhibitor was administered in drinking water (100 mg/kg per day) for 5 days prior to sacrifice. Previous studies have shown this treatment to be highly effective in increasing by five to ten-fold renal renin mRNA accumulation [16, 17]. Care of the mice used in these experiments met or exceeded the standards set forth in *Guidelines for care and use of experimental animals*. Procedures employed were approved by the University Animal Care and Use Committee at the University of Iowa.

Timed pregnancies and recovery of fetuses. Timed pregnancies were set up by breeding female HuRen transgenic mice to male HuRen transgenic mice. Female mice were visually inspected for a vaginal copulation plug and the date of the plug was recorded. The detection of a vaginal plug corresponded to 0.5 days of gestation (dg). One pregnant mouse each was sacrificed on 15.5 and 17.5 dg and the last was allowed to give birth. Newborn pups were sacrificed at approximately 12 h of age. Tissues from the adult mothers of the 15.5 (P_1), 17.5 (P_2) and newborn (P_3) litters were also saved as a control.

Fetuses and newborns were dissected under phosphate-buffered saline using a Nikon SMZ-2T dissecting microscope. Tissues were pooled from the entire litter because of their small size. Kidneys (free of adrenal glands), gastrointestinal (GI) tract, liver, lung, heart and brain were collected. Since both parents were transgenic, 75% of the pups on average should also be transgenic. We anticipated approximately 25% of the pooled tissues to be non-transgenic. We did not expect this to interfere with the detection of human renin. The actual percentage of positive transgenic pups was determined by polymerase chain reaction (PCR) of DNA isolated from placenta (15.5 dg – 100%) or tail (17.5 dg – 63% and newborn – 80%) DNA. DNA from placenta and tail was isolated by digestion of the tissue with proteinase K, followed by RNase A treatment, high salt precipitation of proteins and ethanol precipitation of nucleic acids.

PCR was carried out in a Perkin-Elmer 480 Thermal Cycler using reagents purchased from and the procedure recommended by Cetus. The optimal magnesium chloride concentration was determined empirically and found to be 2.0 mM. Approximately 100 ng of each primer (TGACACTGGTTCGTCCAATG & ATAGCGGAGGGTGAGTTCTG) was included in each reaction which was cycled 35 times by incubating the reaction for 1 min at 94° C, 1 min at 55° C and 1 min at 72° C. Each reaction contained 0.2% of the total DNA extracted from tail or placenta and was estimated to be approximately 25–50 ng. PCR products were visualized by gel electrophoresis through a 1.5% agarose gel and stained with ethidium bromide. Mouse renin sequences were not amplified under these conditions (Fig. 2).

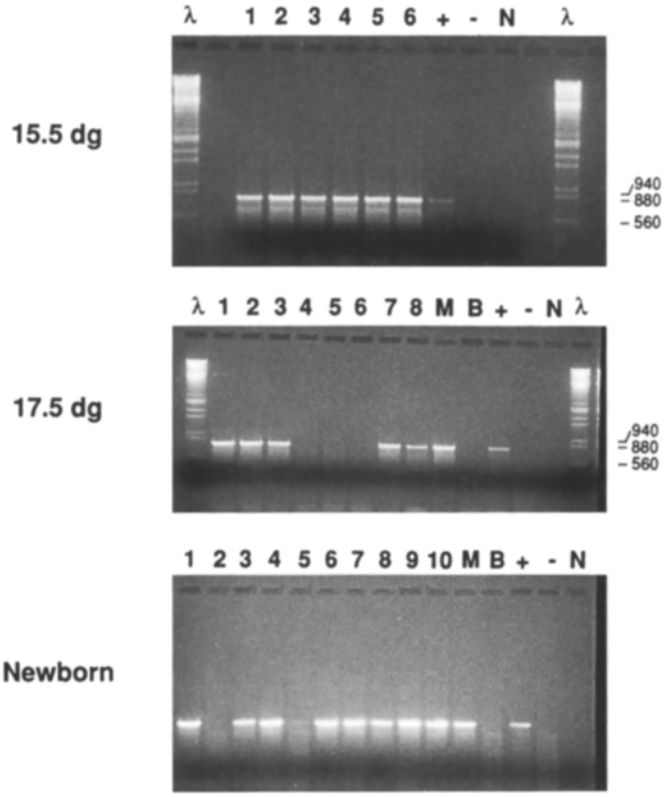

Fig. 2. Identification of transgenic offspring by PCR. PCR was used to identify the number of transgenic offspring making up each pool of tissues. A positive transgenic animal is denoted by having a 754-base pair band. Samples labeled as numbers are offspring while those labeled as *M* are the female parent (mother) of that litter. *B*, Non-transgenic C57BL/6 mice; +, mouse known to be transgenic based on Southern blot analysis and transmission of the transgene to progeny; –, mouse known to non-transgenic based on Southern blot analysis and non-transmission to progeny; *N*, PCR reaction without added DNA; λ, phage lambda DNA digested with *Hin*dIII and *Hin*dIII+*Eco*R1 as markers (the size of several of the marker bands are identified); *15.5 dg*, 15.5 days of gestation; *17.5 dg*, 17.5 days of gestation

Summary of animals used in this study. Tail DNAs from six 15.5 gd fetuses, eight 17.5 gd fetuses and ten newborns were obtained. Controls included tail DNAs from definitive transgenic and non-transgenic adults, the mother of each litter and an adult C57Bl/6 mouse (Fig. 2, 3). Kidney RNAs were from two transgenic and two non-transgenic adults. Adrenal gland RNAs were from an adult transgenic mouse (Fig. 4). Multiple tissues were obtained from fetal transgenics and kidney RNA from the mothers of each litter (Fig. 5). Kidney and lung RNA from fetal and newborn transgenics and adult transgenic and non-transgenic controls were obtained (Fig. 6). Lung RNA was obtained from an adult transgenic mouse (Fig. 7).

Analysis of nucleic acids. Total tissue RNA was isolated and purified by homogenization in guanidine isothiocyanate followed by ultracentrifuga-

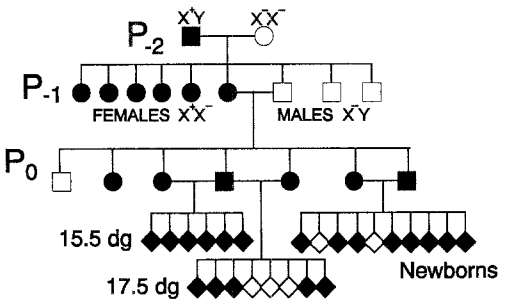

Fig. 3. Summary of breeding results of the HuRen no. 9 line. The parent mice of the fetuses and newborns used in this study are denoted as P_0. Two earlier generations are denoted as P_{-1} and P_{-2} and are used to demonstrate the sex-linked mode of inheritance exhibited by the transgene in this line. □, Non-transgenic males; ■, transgenic males; ○, non-transgenic females; ●, transgenic females; ◇, non-transgenic offspring (not sexed); ◆, transgenic offspring (not sexed)

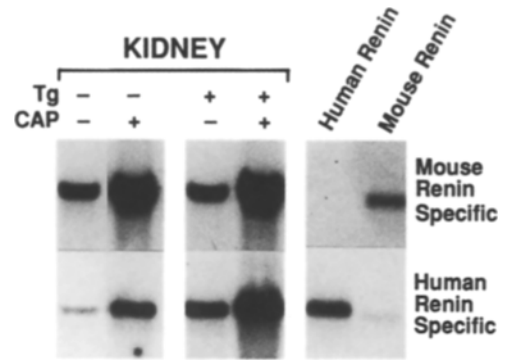

Fig. 4. Expression of human renin mRNA in the adult kidney. Kidney RNA (20 μg) from non-transgenic (*Tg*⁻) or transgenic (*Tg*⁺) kidney was hybridized using probes and conditions favoring the detection of the endogenous mouse renin mRNA (mouse renin specific) or human renin mRNA (human renin specific). The human renin mRNA control (*right*) was from transgenic adrenal gland from mice which do not themselves express adrenal renin. The mouse renin control (*right*) was from DBA/2J adrenal gland. The mice were treated with captopril (*CAP*) in their drinking water for 5 days as indicated. Only adult tissues were used in this experiment

tion through a cesium chloride pad as described [18]. RNase northern blot protection was developed to rapidly assess the relative expression of the human renin gene and to differentiate human renin mRNA from the endogenous renin mRNA in tissues where both are co-expressed, and was performed as previously described [9]. In brief, northern blots were probed with a human renin antisense RNA probe. The human renin clone contains a portion of the complementary DNA (cDNA) spanning a region from the *Eco*R1 site at coordinates 741 to an *Sst*I site at coordinates 1383 of the cDNA sequence and encompassing exons VI–IX cloned into pGEM-3 (Promega). The blots were probed under standard conditions and exposed to X-ray film [18]. To differentiate the mRNAs, the blots were washed in 2X SSC containing 1.0 μg/ml RNAse A for 15 min at 25° C followed by a stringent wash in 0.1X SSC, 0.1% sodium dodecyl sulfate for 20 min at 65° C. This treatment destroys the hybridization between an RNA probe and an RNA immobilized on a filter when they exhibit a large number of mismatches, as they do when the human renin probe is hybridized to endogenous mouse renin mRNA, or vice versa. Without the RNAse treatment, the human renin probe cross-reacts with both the human and mouse renin mRNA. However, after the RNAse treatment, approximately 90% of the sequences detected using the human probe are human renin mRNA, the other 10% being residual hybridization to mouse renin mRNA. The mouse renin probe has been described previously [17].

The differential primer extension assay was also previously described [9]. In short, total RNA is hybridized to a ^{32}phosphorus-end-labeled 37-base oligonucleotide containing the sequence 5′-GACTTT-GAAGGTCTGGGGTGGGGTGCCGATGCCAATC-3′. The hybrids are then used as a template for primer extension by incubating them in the presence of AMV reverse transcriptase and 200 μM deoxycytidine triphosphate, deoxyguanosine triphosphate and thymidine triphosphate, and 200 μM of the chain-terminating nucleotide dideoxyadenosine triphosphate. The buffers and incubation conditions have been described previously [9, 19]. This incubation results in differentially truncated nucleotide chains of 43 (human) and 45 (mouse) bases in length. These products are separated from the unextended oligonucleotide on an 8% acrylamide/8 M urea sequencing gel.

Primer extension sequencing was performed on adult lung RNA using a minor modification of the differential primer extension technique previously described. Lung RNA (40 μg) was hybridized to the end-labeled 37-mer oligonucleotide. The hybrids were divided into four reactions, each containing all four deoxynucleotides at 200 μM and one dideoxynucleotide at 100 μM. Buffers and incubations are as previously described [9, 19]. The sequences were resolved on a 6% polyacrylamide/8 M urea sequencing gel.

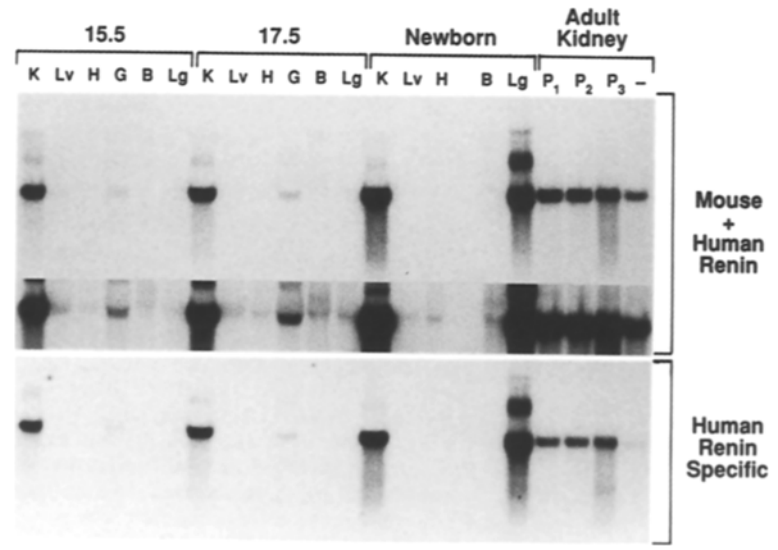

Fig. 5. Expression of human renin mRNA during late fetal development and in newborns. Total tissue RNA (20 μg) from kidney (*K*), liver (*Lv*), heart (*H*), gastrointestinal tract (*G*), brain (*B*) and lung (*Lg*) from 15.5 dg, 17.5 dg and newborn mice was hybridized with probes allowing the detection of total renin mRNA (mouse + human, *top 2 panels*) or specifically human renin mRNA (human renin specific, *bottom panel*). P_{1-3} refers to kidney RNA from the female parents of the 15.5 dg, 17.5 dg and newborns, respectively. Adult kidney RNA from a non-transgenic mousewas also included (–). The *middle panel* is a longer exposure of the top panel

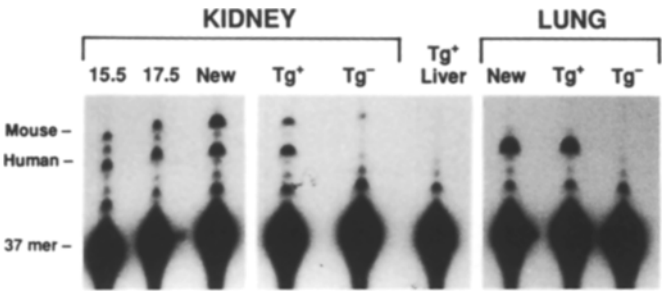

Fig. 6. Differential primer extension analysis. Total tissue RNA (40 µg) was subjected to differential primer extension. The positions of the human- and mouse-specific products are indicated as well as the unextended 37-mer oligonucleotide

Results

Characterization of the HuRen no. 9 transgenic line

The initial characterization of transgenic mice containing the human renin gene has been previously described [9]. Herein we present more detailed information on one of these human renin transgenic lines – HuRen no. 9. A schematic representation of the transgene is presented in Fig. 1. The transgene contains all exon and intron sequences within the human renin gene and includes sequences extending approximately 900 and 400 bp upstream and downstream of the gene, respectively. Timed pregnancies were performed in order to obtain transgenic fetuses at various stages of late murine development. Fetuses (15.5 dg and 17.5 dg) and newborns were recovered and dissected. Tissues were pooled for RNA extraction and placenta and tails were recovered to assess the number of transgenic animals in each pool. Figure 2 shows the results of PCR of DNA partially purified from the placenta of 15.5 dg fetuses and tail of the 17.5 dg fetuses and newborns. The ethidium bromide-stained band migrating at 754 bp is indicative of the presence of the human renin gene in the genome of the mice. The oligonucleotides chosen as primers for this experiment are shown in Fig. 1. These primers each have 6 of 20 bases mismatched with the endogenous mouse renin sequence, rendering the oligonucleotides human renin specific. No amplified product can be seen in the negative control lanes (– or B) containing DNA known to be non-transgenic (Fig. 2). All of six 15.5 dg fetuses (100%), five of eight 17.5 dg fetuses (63%) and eight of ten newborns (80%) were scored as transgenic. This result was better than the 75% success rate expected when breeding a transgenic female with a transgenic male.

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                10         20         30         40         50         60
Human    TCGCCATAGT ACTGGGTGTC CATGTAGTTG GTGAGGATCA CGGAGGAGGT GGTGTTGCCA AG
Ren-1    .....G.... .......A.T ..G....... .......C.. ...G...... AAGA.CAGTC .A
Ren-2    .......... .....C.A.T ..G....... .......C.. ...G....A. AAGA...GTC .A
RAT      ..A....... .......A.. ..A....... .......C.. ...G...... AACA...GT. .A
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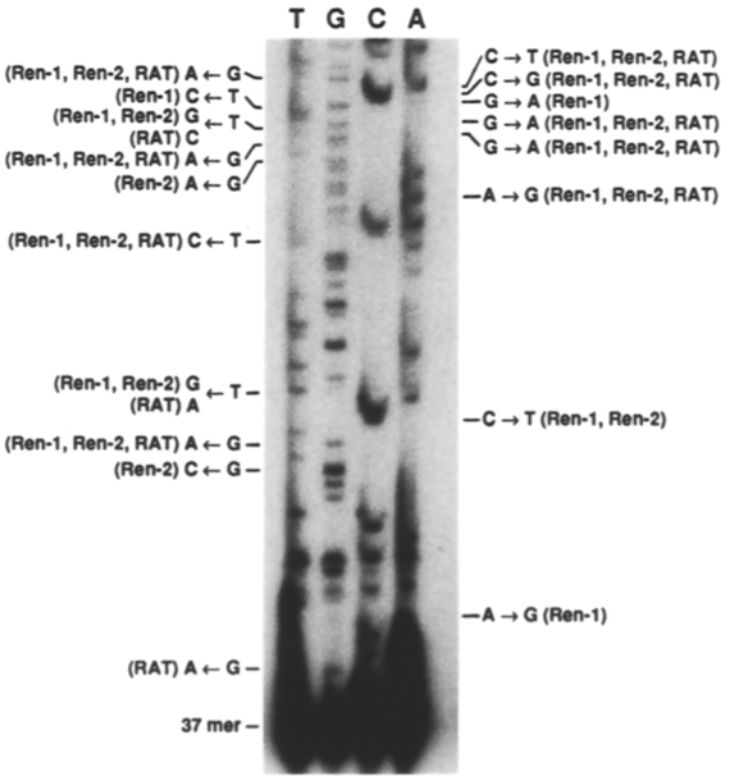

Fig. 7. Primer extension sequencing of adult lung human renin mRNA. Total lung RNA (10 µg) was sequenced in each lane. Differences in sequence between the human renin mRNA and the renin messages of mice and rats are indicated next to the sequencing gel and are summarized at the *top* of the figure. The position of the unextended 37-mer oligonucleotide is indicated

A summary of these results are shown in the pedigree (Fig. 3) which also serves to demonstrate that the transgene in this line is present in the X chromosome and exhibits a sex-linked mode of inheritance; the parents of the fetuses and newborns are labeled as the P_0 generation. Starting two generations back (P_{-2}), a male transgenic crossed with a female non-transgenic resulted in all females but no males being transgenic, strongly indicating that the X chromosome donated by the male parent to the female offspring contained the transgene. All male offspring obtained the Y chromosome from the male parent and the X chromosome from the female parent. Our hypothesis proved to be correct in the P_{-1} generation when a transgenic female crossed with a non-transgenic male resulted in a redistribution of the transgene to both males and females. Although, we did not identify the sex of the fetuses and newborns used in our study, we hypothesize that all non-transgenic pups were male since all females of the P_0 cross ($X^+X^- + X^+Y$) would be transgenic (either X^+X^+ or X^+X^-).

Expression of human renin mRNA in the kidney of adult HuRen no. 9 transgenic mice

Expression of renal human renin mRNA was examined in transgenic line no. 9 adults. Transgenic and non-transgenic mice approximately 8 weeks of age were either left untreated or were administered captopril (100 mg/kg per day) in their drinking water for 5 days. Total RNA was extracted from the kidney tissue and northern blots containing the total RNA were hybridized with antisense mouse renin (Fig. 4, top panel) and antisense human renin (Fig. 4, bottom panel) cDNA probes. The specificity of these probes under the conditions used are illustrated in the right most panel of Fig. 4. Total RNA containing either species of renin mRNA were each hybridized with the species-specific probes. Under our conditions the mouse renin probe only detected the mouse renin mRNA while the human renin probe favored (greater than 10-fold) the detection of the human renin mRNA.

The expression of endogenous mouse renin mRNA was clearly induced several fold after the captopril administration of both non-transgenic (left panel) and transgenic (right panel) mice. Expression of the human renin mRNA was only present in the transgenic animals and was similarly induced. The residual signal in the non-transgenic mice is the result of the human renin probe not being 100% species specific.

Expression of human renin mRNA throughout late ontogeny

Expression of the human renin gene was then assessed in the kidney, liver, heart, GI tract, brain and lung of 15.5 dg, 17.7 dg and newborn mice (12 h of age, Fig. 5). In the top two panels, the blots were probed under conditions in which both human and mouse renin mRNA are detected. The bottom panel was probed with a human renin-specific probe. Expression of human renin was evident in the kidney at all three stages and it appeared to be higher during fetal and newborn life than in the control adult mothers (P_{1-3}). Expression was also visible, albeit relatively low, in the GI tract of the 15.5 and 17.5 dg fetuses. Full length RNA was not recovered from the GI tract of the newborn. No expression was visible in fetal or newborn liver, heart or brain, consistent with the previous results we obtained from adult transgenic mice [9]. The most interesting pattern was observed in lung, where no expression was evident at 15.5 or 17.5 dg but where very high expression was evident in the newborn mice. Although a single predominant species of renin mRNA was observed in kidney, a mixture of mature and longer renin transcripts was evident in the lung. It remains unclear if this represents unprocessed message or message initiated at upstream transcriptional start sites.

The identity of the species of renin mRNA in the kidney and lung of the transgenics was further examined by differential primer extension, an assay capable of differentiating the closely homologous mRNAs (Fig. 6). The results clearly demonstrate that both mouse and human renin are coexpressed in the kidney throughout late development (lanes labeled 15.5, 17.5 and New) as well as in adults (Tg^+). Only the mouse renin gene is expressed in non-transgenic mice (Tg^-) and no renin mRNA was evident in liver. As anticipated from our previous findings of human renin in the adult transgenic lung, the species of renin mRNA detected in the newborn lung is exclusively human [9].

One further test was performed to definitively prove that what was detected in lung was indeed human renin mRNA. This was done by subjecting total RNA extracted from adult transgenic lung to primer extension sequencing. This assay is essentially the same as the assay shown in Fig. 6, except that each reaction contains a small quantity of a dideoxynucleotide along with all four deoxynucleotides. The result is a ladder of bands that can be read as a standard sequence. Figure 7 shows the primer extension sequencing result along with a compilation of sequence polymorphisms between the human renin gene and the renin genes of mice (*Ren-1* and *Ren-2*) and rats. The sequence clearly shows perfect identity with the published human renin cDNA sequence and confirms the results observed by differential primer extension [20–22].

Discussion

Expression of human renin in the HuRen no. 9 line was examined in fetal and newborn mice. Expression of human renin was evident in adult kidney and its expression was inducible after relieving the feedback repression on renin transcription mediated by angiotensin II [23]. In the fetal and newborn mice human renin mRNA was evident in kidney, and in fetal mice it was detectable, albeit at lower levels, in the GI tract. Expression of human renin was high in newborn lung but its expression was undetectable in fetal lung, strongly suggesting that the human renin gene is temporally regulated in this tissue.

The renin gene in mammals exhibits a complicated pattern of tissue- and cell-specific expression. The main site of renin synthesis and regulation is the kidney, where it is

synthesized, stored and released into the circulation by juxtaglomerular cells. These cells have the ability to regulate the level of renin mRNA transcription and renin release over a two-order of magnitude range depending, among others, on the arterial pressure and plasma electrolyte status of the organism. In addition to the kidney, renin is also expressed in a number of other tissues including adrenal gland, ovary, testes, coagulating gland and submandibular gland. Expression of renin at some of these sites is species specific [2, 24]. In addition, renin has been reported but not proven to be expressed in heart, lung, vasculature and brain.

The identification of human renin mRNA in the kidney of adult and fetal transgenic mice, permits us to assess if it undergoes the same developmental changes exhibited by the mouse and rat genes [25–27]. In rodents renin mRNA first becomes detectable in the kidney at approximately 15.5 dg when its mRNA is localized to smooth muscle cells of the developing renal arterial tree. As the arterial tree begins to branch and develop further, renin expressing cells become progressively restricted to the smaller arteries and arterioles. Renin expression finally becomes limited to the juxtaglomerular cells as nephrogenesis continues close to and after birth. Other studies have shown that renal vascular smooth muscle cells, once capable of expressing renin during fetal development, can be induced by a variety of pharmacological and pathophysiological stimuli to reexpress renin [16, 28]. Furthermore, we showed that important regulatory sequences controlling renin gene expression in the fetal, adult and captopril-treated adult mouse kidney lie within the 5′ flanking region of the gene [17]. Our studies on adult transgenic mice demonstrate that human renin is expressed in juxtaglomerular cells and that renal human renin mRNA is induced following angiotensin converting enzyme inhibition. Clearly, in situ hybridization studies on fetal kidney and adult kidney after angiotensin converting enzyme inhibition are an important next step.

Finally, expression of human renin in lung must be examined in more detail. In adult transgenic mice, human renin was found to be expressed in the lungs of three independent transgenic lines, ruling out the possibility that integration site artifacts are responsible for its expression in this organ. However, only a single transgenic line was used in this study to demonstrate the temporal expression. Although the lung has never been considered a site of renin expression it is important to note that renin has been previously reported in human fetal lung and human pulmonary tumors [29, 30]. In both reports, renin was associated with blood vessels, as it is in the developing kidney. In situ hybridization studies must be performed to identify the human renin-expressing cell type in newborn and adult lung. Moreover, it remains unclear if human renin is expressed in normal human lung tissue, and if so, the role it plays there.

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References

1. Fabian J, Field LJ, McGowan R, Mullins JJ, Sigmund CD, Gross KW (1989) Allele-specific expression of the murine *Ren-1* genes. J Biol Chem 64: 17589–17594
2. Sigmund CD, Gross KW (1991) Structure, expression and regulation of murine renin genes. Hypertension 18: 446–457
3. Field LJ, McGowan RA, Dickinson DP, Gross KW (1984) Tissue and gene-specificity of mouse renin expression. Hypertension 6: 597–603
4. Ekker M, Tronik D, Rougeon F (1989) Extra-renal transcription of the renin genes in multiple tissues of mice and rats. Proc Natl Acad Sci USA 86: 5155–5158
5. Dzau VJ, Brody KE, Ellison RE, Pratt E, Ingelfinger JR (1987) Tissue specific regulation of renin expression in the mouse. Hypertension 9 [Suppl 3]: 36–41
6. Catanzaro DF, Mesterovic N, Morris BJ (1985) Studies of the regulation of mouse renin genes by measurement of renin messenger ribonucleic acid. Endocrinology 117: 872–878
7. Dzau VJ, Ellison KE, Brody T, Ingelfinger J, Pratt RE (1987) A comparative study of the distributions of renin and angiotensinogen messenger ribonucleic acids in rat and mouse tissues. Endocrinology 120: 2334–2338
8. Miller CCJ, Carter AT, Brooks JI, Badge RHL, Brammar WJ (1989) Differential extra-renal expression of the mouse renin genes. Nucleic Acids Res 17: 3117–3128
9. Sigmund CD, Jones CA, Kane CM, Wu C, Lang JA, Gross KW (1992) Regulated tissue- and cell-specific expression of the human renin gene in transgenic mice. Circ Res 70: 1070–1079
10. Seo MS, Fukamizu A, Nomura T, Yokoyama M, Katsuki M, Murakami K (1990) The human renin gene in transgenic mice. J Cardiovasc Pharmacol 16 [Suppl. 4]: S8–S10
11. Fukamizu A, Seo MS, Hatae T, Yokoyama M, Nomura T, Katsuki M, Murakami K (1989) Tissue-specific expression of the human renin gene in transgenic mice. Biochem Biophys Res Commun 165: 826–832
12. Fukamizu A, Hatae T, Kon Y, Sugimura M, Hasegawa T, Yokoyama M, Nomura T, Katsuki M, Murakami K (1991) Human renin in transgenic mouse kidney is localized to juxtaglomerular cells. Biochem J 278: 601–603
13. Ganten D, Wagner J, Zeh K, Koeller M, Bader M, Michel JB, Paul M, Zimmermann F, Ruf P, Hilgenfeld U, Ganten U, Kaling M, Bachmann S, Mullins JJ, Murakami K (1992) Species-specificity of renin kinetics in transgenic rats harboring the human renin and angiotensinogen genes. Proc Natl Acad Sci USA 89: 7806–7810
14. Burton J, Quinn T (1988) The amino-acid residues on the C-terminal side of the cleavage site of angiotensinogen influence the species specificity of the reaction with renin. Biochim Biophys Acta 952: 8–12
15. Oliver WJ, Gross F (1966) Unique specificity of mouse angiotensinogen to homologous renin. Proc Soc Exp Biol Med 122: 923–926
16. Gomez RA, Lynch KR, Chevalier RL, Everett AD, Johns DW, Wilfong N, Peach MJ, Carey RM (1988) Renin and angiotensinogen gene expression and intrarenal renin distribution during ACE inhibition. Am J Physiol 254: F900–F906
17. Sigmund CD, Jones CA, Jacob H, Ingelfinger J, Kim U, Gamble D, Dzau VJ, Gross KW (1991) Pathophysiology of vascular smooth muscle in renin promoter-T antigen transgenic mice. Am J Physiol 260: F249–F257
18. Ausubel FM, Brent R, Kingston RE (1989) Current protocols in molecular biology. Wiley, New York

19. Field LJ, Gross KW (1985) Ren-1 and Ren-2 loci are expressed in mouse kidney. Proc Natl Acad Sci USA 82: 6196–6200
20. Hardman J, Hort Y, Catanzaro DF, Tellam J, Baxter JD, Morris BJ, Shine J (1984) Primary structure of the human renin gene. DNA 3: 457–468
21. Imai T, Miyazaki H, Hirose S, Hori H, Hayashi T, Kageyawa R, Ohkubo H, Nakanishi S, Murakami K (1983) Cloning and sequence analysis of cDNA for human renin precursor. Proc Natl Acad Sci USA 80: 7405–7409
22. Soubrier F, Panthier JJ, Corvol P, Rougeon F (1983) Molecular cloning and nucleotide sequence of a human renin cDNA fragment. Nucleic Acids Res 11: 7181–7190
23. Johns DW, Peach MJ, Gomez RA, Inagami T, Carey RM (1990) Angiotensin II regulates renin gene expression. Am J Physiol 259: F882–F887
24. Sigmund CD, Fabian JR, Gross KW (1992) Expression and regulation of the renin gene. Trends Cardiovasc Med 2: 237–245
25. Richoux JP, Amsaguine S, Grignon G, Bouhnik J, Menard J, Corvol P (1987) Earliest renin containing cell differentiation during ontogenesis of the rat. Histochemistry 88: 41–46
26. Gomez RA, Lynch KR, Sturgill BC, Elwood JP, Chevalier RL, Carey RM, Peach MJ (1989) Distribution of renin mRNA and its protein in the developing kidney. Am J Physiol 257: F850–F858
27. Jones CA, Sigmund CD, McGowan R, Kane-Haas C, Gross KW (1990) Temporal and spatial expression of the murine renin genes during fetal development. Mol Endocrinol 4: 375–383
28. Gomez RA, Chevalier RL, Everett AD, Elwood JP, Peach MJ, Lynch KR, Carey RM (1990) Recruitment of renin gene-expressing cells in adult rat kidneys. Am J Physiol 259: F660–F665
29. Taylor GM, Cook HT, Sheffield EA, Hanson C, Peart WS (1988) Renin in blood vessels in human pulmonary tumors: an immunohistochemical and biochemical study. Am J Pathol 130: 543–551
30. Taylor GM, Cook HT, Hanson C, Peart WS, Zondek T, Zondek LH (1988) Renin in human fetal lung: a biochemical and immunohistochemical study. J Hypertens 6: 845–851